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Autoimmune diseases and inflammation

Models of type 1 (autoimmune) diabetes

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Type 1 (autoimmune) diabetes is caused by T-cell autoreactivity resulting in destruction of the insulin-producing pancreatic islet β cells. Its aetiology involves complex interactions between multiple genetic and environmental factors, its pathogenesis involves interactions between many leukocyte subsets and their associated cytokines and its complications affect nearly every organ in the body. Modelling the disease is, therefore, the subject of much effort and surprising ingenuity. In this article, we review established and emerging models of the disease and give an indication of their use for different types of study.

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Autoimmune diabetes is now considered to be a prototypical example of a T-cell-mediated autoimmune disease. In recent years, there has been enormous progress in the analysis of the genetic and immunological mechanisms underlying this disease. In turn, this is leading to several new therapeutic strategies. These efforts have been helped considerably by the convergence of findings from clinical studies and rodent models, especially those based on the nonobese diabetic mouse strain. Here, Alan Baxter – an expert in clinical and experimental models of diabetes – reviews the currently available models.

Introduction

Diabetes associated with insulin deficiency varies widely in its clinical features. A small proportion of cases is characterised by abrupt onset at birth, or in the first few months of life, without good evidence of autoimmune responses. A larger group with atypical diabetes develops the disease later in life, often in middle age, and shows variable insulin dependence and many features of type 2 diabetes. This article is limited to the discussion of models of type 1 (autoimmune) diabetes, which is classically characterised by: (1) onset in childhood, puberty or early adulthood; (2) evidence of autoimmune activation (circulating autoantibodies, T cells, or both, with specificity for islets, β cells or their constituents); (3) severe insulin deficiency associated with low C peptide levels; and (4) if untreated, the biochemical sequelae associated with the resulting failure in glucose homeostasis (polyuria, polydipsia, weight loss, ketoacidosis, coma and death).

Type 1 diabetes

Autoimmune diabetes is a disease of complex aetiology, with large numbers of poorly characterised genetic and environmental risk factors and modifiers. The pathogenesis of the disease is almost certain to be autoimmune in nature because: (1) it is associated with autoimmune phenomena; (2) it showed evidence of immunological memory in diabetic patients that received human leukocyte antigen (HLA)-matched pancreas transplants [1]; and (3) diabetes was inadvertently adoptively transferred by transplantation of non-T-cell-depleted bone marrow [2]. Owing to the relatively inaccessible location of the pancreas and the presence of digestive enzymes in the exocrine tissue of the organ, only limited data are available from biopsies. Similarly, pathological surveys of pancreatic tissue in patients dying of type 1 diabetes are surprisingly scant. As a consequence, the understanding of the mechanisms of β cell destruction is heavily influenced by *in vivo* models of the disease.

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Table 1. Comparison summary table

	In vitro models	*In vivo* models	*In silico* models
Pros	Simple, inexpensive, scalable	Generally the best available	Flexible
Cons	Limited in application	Complex, difficult to control	Dependant on data training
Best use of model	Studies of pathogenesis	NOD mouse: studies of aetiology and pathogenesis (especially genetic and environmental interactions), development of prediction strategies and identification of putative therapeutic interventions. BB rat: physiological studies, testing therapeutic devices. Transgenic: studies of pathogenesis, identification of autoantigens	Prediction
How to get access to the models	Insulinoma available from Edward H. Leiter[a]; TCR transgenic mice available from Kathryn Haskins[b] and Pere Santamaria[c]	NOD mouse available from Jackson laboratory[d]; BB rat available from Taconic[e]; TCR transgenic mice available from Kathryn Haskins[b] and Pere Santamaria[c]	Peptide-binding model available from Vladimir Brusic[f]; generalized linear modelling tools available from SAS[g]
References	[3–8]	[9–23]	[24–27]

Abbreviations: BB, BioBreeding; NOD, nonobese diabetic; TCR, T-cell receptor.
[a] http://www.jax.org/staff/edward_leiter.html.
[b] http://www.uchsc.edu/immuno/faculty.html.
[c] http://www.med.ucalgary.ca/webs/irgtraining/home/.
[d] http://www.jax.org/.
[e] http://www.taconic.com/.
[f] http://sdmc.i2r.a-star.edu.sg:8080/~vladimir/.
[g] http://www.sas.com/.

In vitro models

Although tissue and cell culture systems are unable to model the induction phases of the autoimmune response, they play a major role in the investigation of effector mechanisms of β cell destruction. Cultured insulinoma cells (e.g. NIT-1 [3]) and islets from mouse, rat or human [4] pancreata have been exposed to cytokines and immune effector molecules, such as Fas ligand and nitric oxide, to determine their susceptibility to killing or functional modification by these factors [5]. NIT-1 insulinoma cells can be obtained by contacting the Jackson laboratory (http://www.jax.org/) (Table 1).

An extension of these studies is the co-culture of islets, β cells or antigen-presenting cells pulsed with islet lysates, with various immune cell subsets (usually cloned T cells) from either mouse models or affected patients [6]. These approaches have been used to characterise the immune effector mechanisms of disease [7] and to identify putative autoantigens [8]. Currently, the most convenient source of clonotypic T cells for such studies is T cell receptor (TCR) transgenic mice.

In vivo models

NOD mice

The nonobese diabetic (NOD) mouse strain is the most characterised and best-validated model of autoimmune diabetes; it is the gold standard for modelling aetiological, immunological, pathological and genetic aspects of the disease. The most extensively used line is the NOD/Lt line, which is available from the Jackson laboratory. Both male and female mice of this strain spontaneously develop lymphocytic infiltrates into their pancreatic islets of Langerhans and, depending on housing conditions, up to 90% of female and 50% of male NOD mice succumb to diabetes caused by specific, T-cell-mediated, destruction of the insulin-producing β cells. As in the human disease, many genetic regions (currently more than two dozen) have been linked to diabetes, and environmental factors such as intercurrent infections seem to play a role in modulating tissue destruction, its biochemical sequelae, or both. Perhaps the most extraordinary aspect of the model is that there is some evidence that the same genetic risk factors that contribute to disease susceptibility in NOD mice also do so in humans. Several mouse diabetes loci have been mapped to locations syntenic to human diabetes susceptibility genes (*Idd1*, *Idd4*, *Idd5* and *Idd21*) and, in at least some cases, the homologous gene has been shown to be involved [*A* (GenBank accession numbers NM_010379 and X54425) and *Ctla4* (GenBank accession number NM_009843)] [9]. Indeed, NOD mice have an amino acid substitution at the same position in the gene homologous to the major histocompatibility complex (MHC) class II molecule DQβ, to which much of the HLA-linked disease susceptibility in humans has been attributed [10].

Some concerns have been raised about this model. Islet destruction occurs over a much briefer period in NOD mice than in humans; the infiltrates are much more pronounced in mice; the autoantibody specificities show some differences

[11]; the diabetes is at the severe end of the clinical spectrum; and, if the diabetes is not treated, death tends to occur from dehydration, rather than ketoacidosis. Recent scepticism over failed clinical trials of candidate therapies identified in the mice [12] is probably unwarranted, because, generally speaking, the trial protocols concerned did not compensate for limitations in clinical translation, such as dose escalation and narrow therapeutic windows, that were clearly identified by the preclinical studies.

Transgenic mouse models

One aspect of the complexity of the NOD mouse encompasses the processes required to generate a high frequency of autoreactive T cells bearing receptors with sufficient affinity to cause β cell destruction. A transgenic approach has been taken to eliminate this issue by inducing the transgenic expression of a TCR that is specific for native β cell autoantigens {receptor BDC2.5 – available from Diane Mathis' laboratory (www.hms.harvard.edu/dms/immunology/fac_mathis.html) [13]; receptor BDC6.9 – available from Kathryn Haskins' laboratory (http://www.uchsc.edu/immuno/faculty.html) [14]; receptor 4.1 [15] and receptor 8.3 – available from Pere Santamaria (http://www.med.ucalgary.ca/webs/irgtraining/home/) [15,16]}, by inducing transgenic expression of a non-native antigen on the β cells, for which a high frequency of reacting T cells can be generated {e.g. the nucleoprotein of lymphocytic choriomeningitis virus – available from Matthias Von Herrath's laboratory (http://www.liai.org/) [17]}, or by transgenic expression of both a TCR and a target antigen {e.g. the INS-HA TCR against influenza hemagglutinin under control of the rat insulin promoter – available from Harald von Boehmer (http://www.dana-farber.org/res/departments/cancerimmunology/) [18]}. The inherent simplicity of these models can offer distinct advantages over the NOD mouse in the study of accessory factors (such as molecular mimicry, effects of intercurrent infection, accessory molecules and cytokines) in the pathogenesis of disease. Because the TCRs involved are either CD4 or CD8 associated, it is possible to study the roles of these T cell subsets in isolation or in carefully titrated combinations.

In several other transgenic mouse models, insulin insufficiency is observed following transgenic expression (usually driven by the rat insulin promoter) of proteins in β cells in the absence of any other genetic or immunological manipulation. These particular models are probably artifactual because they do not show evidence of autoimmune causality.

Virally induced mouse models

Some viruses can induce insulin-dependent diabetes in wild-type (i.e. not genetically manipulated) mice. An example is the encephalomyelitis virus variant D, which causes islet destruction by tropism and direct cytotoxic effects. These models do not involve a major autoimmune component (reviewed in [19]).

Humanised mouse models

A concerted multi-investigator effort is underway to develop transgenic and knockout mouse models of the human immune system for the study of autoimmune diseases and transplantation issues. Various components of human responses are being transgenically introduced into mice, including HLA molecules (optimally in association with targeted deletion of native *H2* molecules), TCRs, CD4 and/or CD8, co-stimulator molecules (such as CD80, CD86) and complement inhibitors. Such systems can be used to study thymic selection of autoimmune T cells [20]. In some cases, the aim is to produce a human-like environment to foster the survival or development of human lymphoid cells following adoptive transfer of peripheral blood lymphocytes and/or bone marrow. In these cases, an immunodeficient mouse host (e.g. *scid* or $rag^{-/-}$) is used, often on a NOD background, because of a coincidental accumulation of several immune defects in this strain (for a review of these systems, see [21]).

BioBreeding rats

Like NOD mice, BioBreeding diabetes-prone (BBDP) rats spontaneously develop anti-islet autoantibodies and T-cell-dependent, ketosis-prone autoimmune diabetes. BBDP rats are available from Taconic (http://www.taconic.com/). A major advantage of BBDP rats over NOD mice is their larger size, which facilitates physiological experiments (e.g. draining of lymphatic ducts, intravital monitoring). Disadvantages include the relatively reduced number of immunological reagents (such as monoclonal antibodies and recombinant cytokines), the lack of genetically manipulated (gene-targeted mutant and transgenic) stocks and the presence of a severe T cell lymphopaenia. Comparison with the related, but non-lymphopaenic, BioBreeding diabetes-resistant strain revealed that the lymphopaenia in BBDP rats was largely attributable to a lack of regulatory T cells expressing the ART2 marker. Adoptive transfer of $ART2^+$ cells into BBDP rats prevents the onset of diabetes. An interesting quirk to this model is that diabetes can be induced in BBDR rats by several treatments, including infection with Kilham rat virus and immune activation with polyinosinic-polycytidylic acid [poly(I:C)] (see [22] and references therein).

Streptozotocin-induced diabetes

Streptozotocin is a broad-spectrum antibiotic with diabetogenic properties mediated by direct β cell cytotoxicity [available from Sigma-Aldrich (http://www.sigmaaldrich.com)]. A single dose of 200 mg kg^{-1} results in near-complete elimination of insulin production in a broad range of species, including mice. This model has no major autoimmune features, but is used widely to model the complications of disturbed glucose homeostasis associated with insulin insufficiency. Prior to the introduction of the NOD mouse and BioBreeding (BB) rat, a model of diabetes induced by repeated administration

of lower doses (30–40 mg kg^{-1}) was commonly used, in the belief that the associated islet infiltrates indicated an autoimmune origin for the ensuing β cell loss. Although this model has been largely discredited as a model of autoimmunity, it is still occasionally used in species for which other models are not available [23].

In silico models

Computer modelling of autoimmune processes, and indeed immune responses generally, is currently in its infancy [24]. Perhaps the most comprehensive model of the biochemical pathophysiological processes associated with type 1 diabetes published to date is the Archimedes diabetes model [25]. This model consists of a network of interrelated variables (e.g. plasma insulin level, glucose uptake by muscle and glucose production by liver) linked by differential equations describing the nature of the interactions between each variable. The variables used, and the existence of interactions between them, are selected by the investigators on the basis of current knowledge of biological systems. Output variables can be extremely complex, and include severity of symptoms and the presence of vascular complications. The model requires training on clinical data sets to derive each differential equation. It then iteratively generates a pool of virtual individuals with specific clinical and biochemical characteristics, from which subsets can be selected according to clinical trial criteria. The model provides good correlation with the results of data sets obtained from independent clinical studies. As currently configured, Archimedes does not mimic immune processes but, in principle, these too could be incorporated.

An alternative strategy is the application of a generalized linear model in which outcomes are defined and explanatory variables (risk factors or causes) that might or might not be relevant to the disease processes are nominated [26]. Training on extensive data sets is needed to generate the linear predictor, an equation that contains the explanatory variables, their weightings and arithmetic terms that combine them in additive or synergistic ways, and the transforming link function which maps the output of the linear predictor to an appropriate distribution. An important advantage of this model is that it is not necessary to determine which explanatory variables are important or to understand pathological processes to model them. A major disadvantage is the large size of the data sets required for training. Generalized linear modelling is now commonly used and implemented in many statistical software packages, including SAS (http://www.sas.com/).

Artificial neural networks have been used to model individual components of autoimmune processes. For example, Honeyman *et al.* [27] created a model to predict peptides that bind to the diabetes-associated MHC class II molecule HLA-DR4(*0401) to predict T-cell epitopes of the autoantigen tyrosine phosphatase IA-2 [available from Vladimir Brusic (http://sdmc.i2r.a-star.edu.sg:8080/~vladimir/)]. Predictions were validated by testing synthetic peptides for their binding to DR4 and their ability to stimulate T-cell proliferation in humans at risk for diabetes.

Conclusions

The best models of the complex aetiology of type 1 diabetes are, in order, the NOD mouse and the BB rat. These models are also useful for examining aspects of the pathogenesis of the disease, although many of the transgenic models also make important contributions. Diabetes induced by nonautoimmune processes (such as poisons or viral infection) is only really useful for modelling the complications of hypoglycaemia. Most *in vitro* and *in vivo* models address much more limited questions and are usually adapted to suit the exact requirements of the investigators. The major exceptions to this generalisation are the ongoing attempts to model whole individuals *in silico*, which to date have not successfully simulated immune processes.

The question of how well these models relate to human disease remains an open one. Generally, the study of type 1 diabetes in humans is complicated by the inaccessibility of the affected tissues and a poor association between pathological changes in peripheral blood and those occurring in the islets. Modelling the disease, therefore, has its greatest value in determining the avenues of prediction and intervention that are most worthy of attention in clinical studies.

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